

THE ARBOL DEL TULE
(*TAXODIUM MUCRONATUM* TEN.) IS A
SINGLE GENETIC INDIVIDUAL

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ABSTRACT

El Arbol del Tule (The Tule Tree: *Taxodium mucronatum* Ten. [Taxodiaceae]), found in Santa María del Tule, Oaxaca, Mexico, is considered to have the largest trunk circumference in the world. It has been suggested, however, that this tree may be derived from the fusion of multiple individuals. Using random amplified polymorphic DNA's (RAPD's), we found evidence of genetic uniformity of the Tule tree among branches, indicating that the Arbol del Tule in all likelihood is a single genetic individual. As such, its claim to title of the tree with largest trunk circumference remains unchallenged.

RESUMEN

El Arbol del Tule (*Taxodium mucronatum* Ten. [Taxodiaceae]), localizado en Santa María del Tule, Oaxaca, México, es considerado como el árbol con el tronco más ancho del mundo. Sin embargo, se ha sugerido que este gigantesco árbol podría haberse formado por la fusión de varios individuos. Utilizando polimorfismos de DNA amplificadas al azar (RAPD's) obtuvimos evidencia de uniformidad genética entre las ramas principales del Arbol del Tule, indicando que este árbol está compuesto de un solo individuo genéticamente. Por lo tanto, el título de el árbol con el tronco más ancho del mundo, para el Arbol del Tule, permanece latente.

The intimate association between humans and nature can be identified in the cultural expressions of most civilizations. Plants, especially those with peculiar features, are closely linked to civilizations in southern Mexico. Such is the case of El Arbol del Tule (the Tule Tree: *Taxodium mucronatum* Ten. [Taxodiaceae]), found in Santa María del Tule, Oaxaca, México. This tree has the largest stem circumference (45.7 m dbh) in the world. Because of its majestic appearance this individual has played an important role in the history and culture of the local region in southern Mexico. *Taxodium mucronatum* is an abundant tree, 10–30 m tall, commonly growing along ravines and small streams. Its abundance and wide distribution in Mexico is reflected in the numerous common names applied to this plant: “ahuehuete,” “ciprés,” “pénjamu,” “sabino,” among

others (Standley 1922; McVaugh 1992). Besides the Tule tree, there are other individuals of this species that presided over relevant historic events, such as "El Arbol de la Noche Triste" (The Tree of the Sad Evening) found near Mexico City. According to some narratives, Cortés cried at the base of this tree after being defeated by the Mexica Indians in 1520 (Alvear 1976).

Hall et al. (1990) wrote a detailed literature review of the historic significance of the Tule tree and discussed the different types of studies undertaken on this tree. It has been suggested that the Tule tree may in fact represent the fusion of multiple individuals (Hall et al. 1990). Trees composed of different individuals have been reported in nature. One of the more striking examples is in the genus *Ficus* (Moraceae). Thomson et al. (1991) found that single trees of strangler figs in Panama, include multiple genotypes, and they are formed by the fusion of different individuals. The aggregate tree hypothesis of the Tule tree has been recently tested by an isozyme analysis of the main branches of this historic tree (Hall et al. 1990). Of the 16 loci surveyed in that study, all except *Skdh-2* were monomorphic, even in two neighboring trees. In addition, all samples of the Tule tree were heterozygous for *Skdh-2* (with *Skdh-2ab*) whereas both neighboring trees possessed only *Skdh-2b*. They concluded that heterozygosity of all the main branches of the Tule tree for *Skdh-2b* contrasting with the homozygosity of the two neighboring trees for this locus provides strong evidence to support the hypothesis that the Tule tree originated from a single seedling. However, the low number of loci available for comparison did not rigorously support the hypothesis that the Arbol del Tule is in fact a single tree.

Random amplified polymorphic DNA (RAPD's) has been successfully used in a great variety of applications in plants such as genetic mapping (Fauré et al. 1993; Nelson et al. 1993; Quiros et al. 1991), analysis of genetic variation (Tao et al. 1993; Wolf and Peters-Van Rijn 1993; Yu and Nguyen 1994), breeding system studies (Fritsch & Rieseberg 1992), DNA fingerprinting (Adams et al. 1993; Rieseberg et al. 1994), parentage determination (Welsh et al. 1991) and identification of molecular markers linked to specific traits (Martin et al. 1991; Chalmers et al. 1993), among others. Using RAPD's, we tested the hypothesis of genetic unity of the Tule tree. If all the main branches of this tree are identical at all RAPD loci, then the Tule tree is in all likelihood a single genetic individual. In contrast, the discovery of RAPD polymorphisms among branches of the Tule tree would support the multiple genotype hypothesis. However, for this to be a valid test of these alternative hypotheses, it is necessary to demonstrate that sampled loci are polymorphic in natural populations of *Taxodium mucronatum*. Thus, we also compared the results from the Tule tree with variation in two wild pop-

TABLE 1. POPULATIONS OF *TAXODIUM MUCRONATUM* ANALYZED FOR RAPD VARIATION. Vouchers are deposited at the herbarium of the University of Morelos (HUMO).

| Population designations | Locality and collection number |
|-------------------------------|--|
| Tule Tree | Santa María del Tule, Oaxaca, México, <i>Dorado 2305, Cota and Leyva.</i> |
| “Tule brothers” CUERNAVACA | Same locality, <i>Dorado 2306, 2307, Cota and Leyva</i> Col. Rancho Tetela, Cuernavaca, Morelos, <i>Dorado 2308, Ramírez, and Flores.</i> |
| TEMIXCO | Puente del Diablo, 2 km al N de Temixco, Morelos, <i>Dorado 2309, Ramírez and Flores.</i> |

ulations of the same species, as well as with nearby individuals in Santa María del Tule.

MATERIALS AND METHODS

Fresh leaf tissue was collected from the six main branches of the Tule tree of Santa María del Tule, Oaxaca, Mexico. In order to detect any possible variation we sampled from both nearby populations and more distant populations. Two individuals of *Taxodium mucronatum* found in close proximity (ca. 30 m) were collected, and they were designated in this study as “Tule brothers” samples 7 and 8, respectively. In addition, 15 individuals in each of two distant populations in Cuernavaca and Temixco were sampled to assess levels of RAPD variation within wild populations of this species (Table 1). Leaves were stored on ice and total DNA was isolated from 30 mg of leaf tissue from each sample. DNA isolations followed Doyle and Doyle (1987) with three modifications. First, all volumes were reduced to allow isolations to be performed in 1.5 ml microfuge tubes. Second, sodium metabisulfite (1% w/v) was added to the CTAB buffer. Third, two chloroform extractions were performed.

Primers known to amplify strongly in plants (Fritsch et al. 1993) were used for amplification. Primers were obtained from Operon Technologies (Alameda, California) and University of British Columbia Biotechnology Center. Amplification conditions followed Williams et al. (1990) and were carried out in a MJ Research thermal cycler (Watertown, MA). Amplified products were electrophoresed through 1.5% agarose gels using TBE buffer, stained with ethidium bromide, and photographed using a UV light source. In order to standarize amplification conditions, DNA isolates were quantified with a fluorometer (Hoefer), and standardized to 10 ng/ μ l. We adopted a conservative approach of scoring only strongly amplified fragments.

A preliminary survey of primers in search of consistent and reliable RAPD amplifications was undertaken using five branches

from a single tree from the Cuernavaca population. Of the 104 primers analyzed, 23 primers gave uniform amplifications of all five DNA samples (Table 2). This trial was repeated with five branches from a single tree of the Temixco population. The same 23 primers were again indicated, and these primers were selected for use in the final analysis of the Tule tree and the Cuernavaca and Temixco populations.

RESULTS AND DISCUSSION

A total of 149 fragments was generated, each of which was presumed to represent a locus. Of these fragments, 31 (22.8%) were polymorphic at least within one population (Table 2). However, these polymorphisms were detected only in the Temixco and/or Cuernavaca populations. None of the six branches of the Tule tree showed variation at any of the 149 RAPD loci analyzed. An example of the amplified products from UBC primer 103 is shown in Figure 1. This primer generated variable fragments in several individuals, but the six branches of the Tule tree were invariant.

Although the Tule brothers were similar to the Tule tree, they possessed 15 loci that were different with respect to the Tule tree. In addition, 12 loci distinguished the two Tule brothers from each other. Tule brother 7 differed from the Tule tree by 10 loci, whereas 12 loci distinguished Tule brother 8 from the Tule tree. Thus, Tule brother 7 is slightly more similar to the Tule tree than to Tule brother 8, despite its close geographical distance to Tule brother 8 (ca. 5 m) and its larger distance to the Tule tree (ca. 30 m).

The lack of polymorphism found among the six branches of the Tule tree strongly supports the hypothesis that this gigantic tree represents a single genetic individual. If the Tule tree were in fact a population of several individuals, it seems likely that some of the 23 primers and 149 loci (31 polymorphic) would show variation, as they did in the Cuernavaca and Temixco populations, and as they did in the Tule brothers.

It may be argued, given the nature of cone dispersal in *Taxodium*, that a single cone may give rise to several siblings and that the Tule tree is an aggregate of siblings. However, *Taxodium* is wind pollinated, sexually reproducing, and monoembryonic (Vasil and Sahni 1964), and the ovules of a single cone would likely be, fertilized by genetically different pollen parents. That no genetic variation was detected in the Tule tree suggests that the tree is a single individual not a population of siblings.

Somatic mutation, if it exists in the Tule tree, must surely exist at a lower level than the variation found among younger individuals in the Cuernavaca and Temixco populations. The fact that we found

TABLE 2. NUMBER OF POLYMORPHIC FRAGMENTS IDENTIFIED BY EACH PRIMER IN THE ANALYSIS OF THE ARBOL DEL TULE. PRIMERS A-F ARE FROM OPERON TECHNOLOGIES; I01, I03 AND I06 ARE FROM UNIVERSITY OF BRITISH COLUMBIA BIOTECHNOLOGY CENTER.

| Population | Primer | | | | | | | | | | | | | | | | | | | | | | | | |
|------------|--------|----|-----|-----|-----|----|----|-----|-----|----|----|-----|-----|-----|----|----|----|-----|-----|-----|-----|-----|-----|---|---|
| | A1 | A8 | A20 | B12 | B18 | C1 | C2 | C11 | C19 | D3 | D7 | E18 | E19 | E20 | F4 | F5 | F6 | F10 | F11 | F19 | I01 | I03 | I06 | | |
| Tule | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Cuernavaca | 1 | 3 | 2 | 1 | 0 | 0 | 3 | 1 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 3 | 3 |
| Temixco | 0 | 2 | 1 | 2 | 1 | 1 | 4 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 1 | 3 | 2 | 0 | 1 | 0 | 1 | 0 | 1 | 2 | 1 |

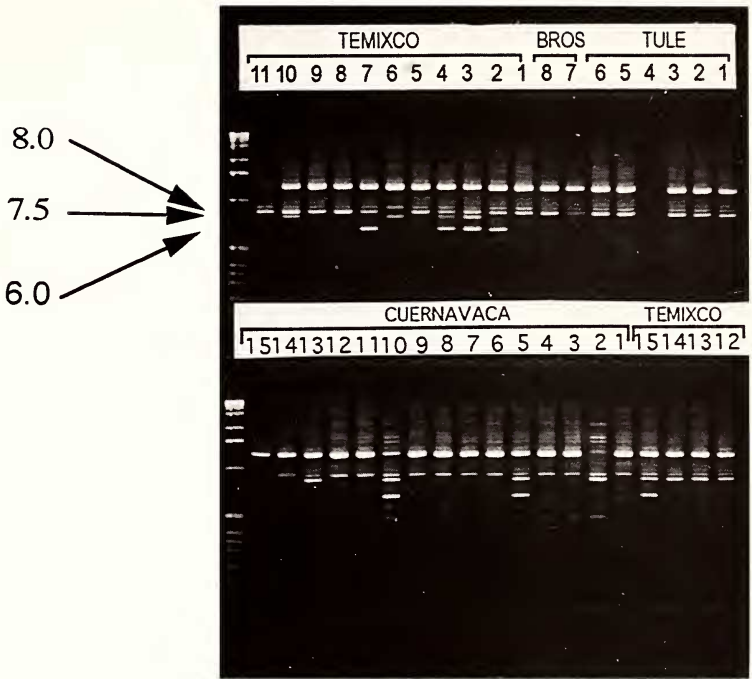


FIG. 1. Electrophoretic patterns of the RAPD products of UBC103 for the Tule tree (lanes 1–6), “Tule brothers” 7 and 8 (lanes 7–8), Temixco population (lanes 9–22), and Cuernavaca population (lanes 23–38). The fragments 8.0, 7.5 and 6.0 kb are polymorphic for several individuals of the Temixco and the Cuernavaca populations. The standard molecular marker (kb ladder: Gibco BRL) is in the last lane of both gels.

no variation *at any level* in the Tule tree samples strongly supports the conclusion that the Tule tree is a single genetic individual.

The results described in this paper show that RAPD markers are a powerful tool for the characterization of single individuals. Our data confirm that the Tule tree is a single, genetically uniform individual. As such, its claim to the title of tree with the largest trunk circumference in the world is unchallenged, and its significance to the people of Santa María del Tule is undiminished.

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LITERATURE CITED

- ADAMS, R. P., T. DEMEKE, and H. A. ABULFATLIN. 1993. RAPD DNA fingerprinting and terpenoids: clues to past migrations of *Juniperus* in Arabia and east Africa. *Theoretical and Applied Genetics* 87:22–26.
- ALVEAR, A. C. 1976. *Historia de México*. Editorial Jus. México D.F.P. 138.
- CHALMERS, K. J., U. M. BARUA, C. A. HACKETT, W. T. B. THOMAS, R. WAUGH, and W. POWELL. 1993. Identification of RAPD markers linked to genetic factors controlling the milling energy requirement of barley. *Theoretical and Applied Genetics* 87:314–320.
- DOYLE, J. J. and J. L. DOYLE. 1987. A rapid DNA isolation procedure for small amounts of fresh leaf tissue. *Phytochemical Bulletin* 19:11–15.
- FAURÉ, S., J. L. NOYER, J. P. HORRY, F. BAKRY, C. LANAUD, and D. GONZÁLEZ DE LEÓN. 1993. A molecular marker-based linkage map of diploid bananas (*Musa acuminata*). *Theoretical and Applied Genetics* 87:517–526.
- FRICTSCH, H. P., M. A. HANSON, C. D. SPORE, P. E. PACK, and L. RIESEBERG. 1993. Constancy of RAPD primer amplification strength among distantly related taxa of flowering plants. *Plant Molecular Biology Reporter* 11:10–20.
- FRICTSCH, P. and L. H. RIESEBERG. 1992. High outcrossing rates maintain male and hermaphrodite individuals in populations of the flowering plant *Datisca glomerata*. *Nature* 359:633–636.
- HALL, G. H., G. M. DIGGS, D. E. SOLTIS, and P. S. SOLTIS. 1990. Genetic uniformity of El Arbol del Tule (The Tule Tree). *Madroño* 37:1–5.
- MARTIN, G. B., J. G. K. WILLIAMS, and S. D. TANSKLEY. 1991. Rapid identification of markers linked to *Pseudomonas* resistance gene in tomato by using random primers and near isogenic lines. *Proceedings of the National Academy of Sciences of the USA*, 88:2236–2240.
- MCVAUGH, R. 1992. Taxodiaceae Pp. 104–106, in W. R. Anderson (ed.) *Flora Novogaliciana: Gymnosperms and Pteridophytes* V. 17.
- NELSON, C. D., W. L. NANCE, and R. L. DOUDRICK. 1993. A partial genetic linkage map of slash pine (*Pinus elliotti* Engelm. var. *elliottii*) based on random amplified polymorphic DNAs. *Theoretical and Applied Genetics* 87:145–151.
- QUIROS, C. F., J. HU, P. THIS, A. M. CHEVRE, and M. DELSENY. 1991. Development and chromosomal localization of genome-specific markers by polymerase chain reaction in *Brassica*. *Theoretical and Applied Genetics* 82:627–632.
- RIESEBERG, L. H., G. J. ALLAN, Y. NUI, J. GREENE, P. MORRELL, S. C. SPENCER, and B. O'BRIEN. 1994. Genetic fingerprinting of various native California cultivars. *Madroño* 41:30–39.
- STANDLEY, P. C. 1922. Trees and shrubs of Mexico. *Contributions of the U.S. National Herbarium* 23:466–470.
- TAO Y., J. M. SANNERS, M. M. LUDLOW, and R. G. HENSELL. 1993. DNA polymorphisms in grain sorghum (*Sorghum bicolor* (L.) Moench). *Theoretical and Applied Genetics* 87:14–320.
- THOMSON, J. D., E. A. HERRE, J. L. HAMRICK, and J. L. STONE. 1991. Genetic mosaics in strangler fig trees: implications for the tropical conservation. *Science* 254:1214–1216.
- VASIL, V. and R. J. SAHNI. 1964. Morphology and embryology of *Taxodium mucronatum* Tenore. *Phytomorphology* 14:369–384.
- WELSH, J., R. J. HONEYCUTT, M. MCCLELLAND, and B. W. S. SOBRAL. 1991. Parentage determination in maize hybrids using arbitrarily-primed polymerase chain reaction (AP-PCR). *Theoretical and Applied Genetics* 82:473–476.
- WILLIAMS J. G. K., A. R. KUBELIK, J. K. LIVAK, J. A. RAFALSKI, and S. V. TINGEY. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18:6531–6535.
- WOLF, K. and J. PETERS-VAN RIJN. 1993. Rapid detection of genetic variability in

- chrysanthemum (*Dendranthema grandiflora* Tzvelev) using random primers. *Heredity* 71:335–341.
- YU, L. X. and H. T. NGUYEN. 1994. Genetic variation detected with RAPD markers among upland and lowland rice cultivars (*Oriza sativa* L.). *Theoretical and Applied Genetics* 87:668–672.

NOTEWORTHY COLLECTIONS

ARIZONA

ANTHOCEROS AGRESTIS Patton (Anthocerotae).—Pinal Co., Tonto National Forest, north of Hwy 60 at Top-of-the-World, between Superior and Globe. T1S R13E, ca. 111°00'40"W, 33°24'N, growing in Mule Spring drainage, west of Power's Gulch in a wet seep area along the stream with mosses, liverworts, and orchids, 30 April 1995, J. Rebman *et al.* 2906 (ARIZ, ASC, ASU, DES, SD).

Previous knowledge. Known to be widespread but more common in the eastern parts of the United States where it can occur as a weed in cultivated areas. This species of hornwort is not well understood and does have nomenclatural problems. The taxon was long known as *A. crispulus*, but the type specimen proved to be a different race of *A. punctatus*; more recently it was placed in *Aspiromitis punctatus* var. *agrestis*, but this name is incorrect based on a typification error (Alan Whittemore personal communication 1995).

Significance. First confirmed record for Arizona and not common in the southwestern USA. The locality where this hornwort was found is part of the proposed Carlota Copper Mine project and may be destroyed as development occurs.

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